Pharmacokinetics, Chemical Interactions, and Toxicological Risk Assessment in Perspective

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Chemical mixtures and multiple routes of exposure are frequently difficult problems for exposure and risk assessors. Chemicals can interact synergistically or antagonistically at a variety of physiologic and biochemical loci within target cells. Many of these interactions can be accounted for with a thorough understanding of the pharmacokinetics of the compounds in the mixture. Many pharmacokinetic processes such as metabolism and absorption can be impacted by the presence of other chemicals in the environment and diet and as a result of medication. In addition, variations between responses as a result of different exposure scenarios (route of exposure, frequency, magnitude) can sometimes result from the impacts upon the pharmacokinetics. Pharmacokinetic models, when properly formulated and tested, can be useful tools to describe and predict the magnitude of the impact of multichemical and multiroute exposures. Several examples will be used to demonstrate this potentially powerful tool and how it can impact the risk assessment process.—Environ Health Perspect 102(Suppl 9):133–137 (1994)

Key words: pharmacokinetics, risk assessment exposure scenarios, relative risk

Introduction

Risk management is a complex process performed in part by comparing and contrasting various risks. Risk assessments should be designed to provide the necessary accurate information to perform these comparisons and contrasts. Incomplete risk assessments effectively become risk management decisions. Such risk management decisions may not always lead to the best long term policies for nations and even the world at large.

Incomplete risk assessments can take many forms. A risk assessment that ignores key scientific data is an obvious example of one that could lead to risk-management decisions that are seriously deficient. Other more subtle forms of incomplete risk assessment also exist. Risk assessments that do not examine the impact of all the different, but realistic, exposure conditions are woefully inadequate. Those that are not able to at least examine the potential for chemical interactions, the reality of most human exposure scenarios, may be equally inadequate.

Traditionally, many risk assessments have been performed using worst case scenarios in both the hazard and exposure assessments. Conventional wisdom has told us that such risk assessments protect, by default, all members of the society. Risk assessments performed exclusively in this manner provide the risk manager with no idea as to the actual number of persons at such worst case levels or who those persons may be. Can those at greatest risk be identified? Can suitable protection be provided for them without necessarily subjecting the entire population to unnecessary and costly protection? Would we, for example, not allow vaccination against a lethal disease because a very small percentage of the population is dangerously sensitive to the vaccine? The answer is clearly no. Our approach is to identify those at unusually high risk and protect those individuals in some special manner, while allowing the rest of the population to be vaccinated. Given the economic and global pressures that nations face today, such multitiered risk assessment methodologies are needed in the area of chemical risk assessments.

Risk assessments are generally thought of as being composed of several components including the hazard assessment and the exposure assessment. Given recent advances (*1,2*), risk assessment should also include the dose assessment as an important component. Dose assessment would include estimates of internal doses resulting from exposure at various conditions. Internal organ or cell level doses are generally considered to be more relevant measures of dose than either exposure or applied dose. With limited knowledge about the mechanism of action, the exact internal dose which will serve as the most toxicologically relevant measure cannot always be known with absolute certainty. This is a situation in which several relevant scenarios must be tried. For example, the mechanism of toxic action may depend upon either the total amount of metabolic transformation or the amount of one specific metabolite interacting with intracellular organelles. In such cases, the assessor must find ways to estimate both of these putative internal doses and compare the impacts of exposure conditions on each. In other cases the complexities are far greater. Different exposure patterns may or may not impact the relative amounts of various metabolites being formed. For example, under a specific exposure pattern greater or lesser ratios of a toxic to a nontoxic metabolite may be formed when compared to a different, yet equally relevant, exposure pattern. In other cases toxicity may result from delicate synergism between parent compound and one or more metabolites. Differences in exposure patterns can result in measurable differences in levels of these synergistically acting relevant toxins in the body.

A better understanding of doses and the relevant interaction of various substances within the body can be gained through the use of physiologically based pharmacokinetic (PBPK) and pharmacodynamic (PBPD) models. This article will illustrate how PBPK models can be used to help make more rational decisions when comparing risks resulting from different exposure conditions.

Models

Two PBPK models are used in this exercise. They each are structured somewhat

This article was presented at the IV European ISSX Meeting on Toxicological Evaluation of Chemical Interactions: Relevance of Social, Environmental and Occupational Factors held 3–6 July 1992 in Bologna, Italy.

This research has been funded wholly by the United States Environmental Protection Agency. The information in this document has been subjected to Agency review and approved for publication.

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differently depending upon the exact modes of toxic action assumed for each case. The models were developed from a combination of two other models published for specific chemicals. One, published in 1990 (*3*) describes the disposition of chloroform in the body. The other, first presented in 1985 (*4*) and discussed in more detail in 1992 (*5*), describes the disposition of 2,5-hexanedione within specific intracellular loci of the brain. The two models developed for this exercise, while not at this time intended for any particular chemical, are actually based on components and characteristics of the chloroform and hexanedione models. Like all PBPK models, these are systems of mass-balance equations describing the disposition of the parent compound within the body and, when so desired, its metabolites. Metabolism is very important in describing pharmacokinetics because it is often a major mechanism for clearing chemicals from the body. It is equally important, and increasingly more obvious, that metabolites are also often found to be the toxic species causing some of the clinical and subclinical adverse endpoints.

The general macroscopic or organ-level characteristics of each model are the same. The models represent the body as being composed of blood, lungs, liver, fat, kidney, and two lumped compartments called the rapidly and slowly perfused tissues. These latter two compartments represent those organs which are not explicitly defined by separate equations. Equations are also included to account for complete mass balance for the parent compound. The mass-balance equations then also give the value of the total absorbed dose in the body. Total absorbed dose is often used in conventional risk assessments. Although based on the two models cited (*3–5*), these models are somewhat uniquely structured. Both models allow for varied and multiroute exposure scenarios. Both are capable of simulating continuous, intermittent, and single exposures by inhalation, zero, and first-order absorption through the gastrointestinal tract. Although intended at the present time to track internal disposition of only one chemical and its metabolites, modifications can be made to consider simultaneous exposure to more than one chemical. As will be discussed, the current models can account for some aspects of simultaneous exposure.

Systems of ordinary differential equations which typically make up PBPK models are solved using numerical techniques. A variety of simulation programs can be written and simulation languages are also commercially available. Regardless of the simulation language or program used, experience has shown that many of these models are composed of differential equations under stiff conditions. Thus, the integration techniques for solving equations at such stiff conditions are necessary. Examples of such solution techniques are Gear's method (*6*) and the Lawrence Livermore Solver of Differential Equations (*7,8*).

Figure 1 shows an overview of both models. The parent chemical can enter the body through the lungs and the gastrointestinal tract. Clearance is through the lungs and through metabolism, occurring in the liver and kidney. Chemical is transported to each organ through the arterial blood and what remains in the blood after extraction by the organ is transported from the organ by the venous return.

The first model used here, termed model 1, is based on the assumption that the particular chemical of interest exerts its toxicity as a result of a very active metabolite, which upon formation in the liver, binds immediately with endogenous hepatic components. It obviously follows that the toxicity caused as a result of this binding occurs at the site of metabolite formation, in this case the liver. The model is structured to account for the cumulative amount of metabolite produced and the cumulative amount bound to endogenous components and molecules. It is this amount of bound metabolite that is considered to be toxicologically relevant and of interest for risk assessment. The parent compound, its metabolism in other tissues, and interaction between parent and metabolites can also, if necessary, be considered for risk-assessment purposes and accounted for in these models.

The second model, termed model 2, assumes that slightly different mechanisms result in toxicity. While the overall macroscopic structure is the same, in this model the metabolite formed can either bind with endogenous ligands or be cleared from the liver by other pathways. The bound fraction then incorporates under first-order kinetic conditions into the endogenous components such as protein or intranuclear materials. The concentration of metabolite incorporated is influenced by the formation process and a concurrent decomposition process. The decomposition can be the result of turnover processes within the tissues, such as protein turnover or cellular death and removal. In this exercise, the model simulations for the concentration of incorporated fraction and the cumulative area under the curve of incorporated fraction versus time, are tracked and examined under various exposure conditions. These have been selected, for illustrative purposes, to be the toxicologically relevant measures of dose.

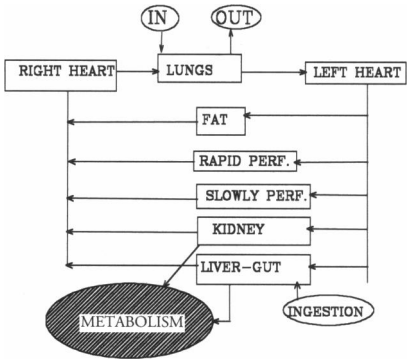

Figure 1. Diagrammatic representation of models.

Scenarios Compared

This illustrative exercise was designed primarily to compare the toxicologically relevant doses produced among different exposure scenarios. Secondly, comparisons are between cases in which there are and are not concomitant exposures to other chemicals which may modulate certain metabolic and physiologic processes that in turn impact the amount of relevant or effective dose produced in the body.

Five different exposure scenarios are examined. The ambient media exposure concentrations for each were adjusted so that approximately the same absorbed dose resulted from each scenario. For examination of the effect of concomitant exposure, one exposure scenario was chosen. The impact of exposure to more than one substance simultaneously was examined. For this illustration the impact upon two physiologically and biochemically relevant hepatic parameters was examined. Table 1 shows the scenarios examined using each model.

Table 1. Exposure scenarios used for comparisons.

Exposure route	Concentration	Duration or frequency
Inhalation	0.00035 ppm	6 hr
Inhalation	0.013 ppm	10 min
Ingestion	0.0018 mg/l	3 per day, single
Ingestion	0.0054 mg/l	1 per day, single
Ingestion	0.0018 mg/l	1 per day, repeated

Results

Figure 2 shows the total mass of compound absorbed after each exposure scenario. As can be readily observed, the mass absorbed after the exposure periods are complete is almost identical for each. The results are the same whether the simulation was performed using model 1 or model 2.

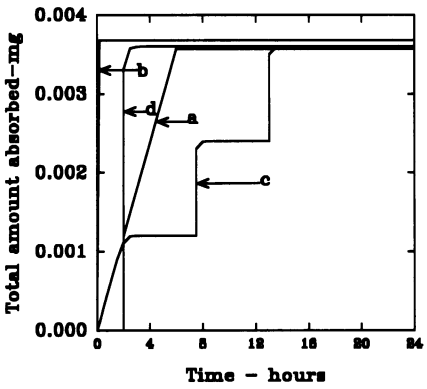

Figure 2. Total mass absorbed as estimated by model 1. (*a*) 6-hr inhalation at 0.00035 ppm; (*b*) 10-min inhalation at 0.013 ppm; (*c*) three repeated ingestion doses at 0.0018 mg/l; (*d*) one ingestion dose at 0.0054 mg/l.

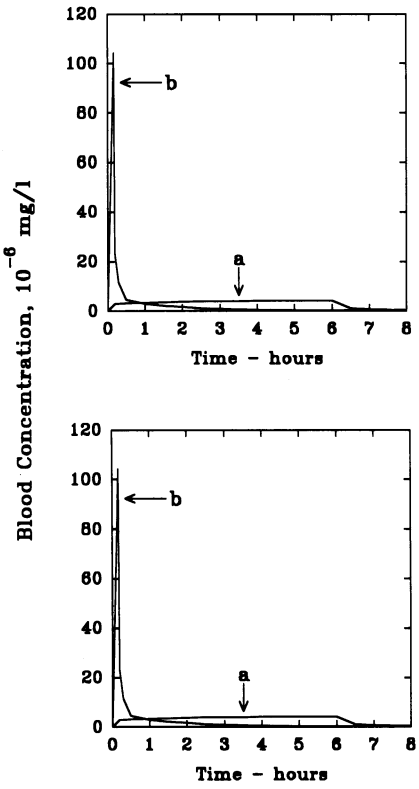

Figure 3. Arterial blood concentration as estimated by model 1. (*a*) 6-hr inhalation at 0.00035 ppm; (*b*) 10 min inhalation at 0.013 ppm; (*c*) three repeated ingestion doses at 0.0018 mg/l; (*d*) one ingestion dose at 0.0054 mg/l.

Figure 3 shows the results of the four simulation scenarios on the arterial blood concentration of parent compound. Obviously, for the arterial blood concentration as an end point of interest, the single high level inhalation exposure provides the greatest dose. The lower arterial concentration after ingestion is due to the first-pass effect exerted by the liver, and described by the model. Model results also indicated that the concentrations in the other organs closely paralleled that of the arterial blood. Thus it can be concluded that, with regard to parent compound concentration peaks, the single high level inhalation scenario gave the greatest dose. If some acute toxic event is assumed to occur after the concentration level reaches a certain threshold, the single level inhalation exposure has the greatest potential to induce deleterious effects.

Different and interesting results are obtained when attention is turned to products of metabolism and interaction of metabolites with endogenous molecules. For these cases, the results are scenario and model dependent. Looking first at the amount of metabolite bound to intrahepatic molecules, Figure 4 shows the results for model 1. Model 1, it should be remembered, accounts for cumulative amount bound from the start of the simulated exposure until the end of the simulation. Hence, the amount bound reaches a plateau. Inspection of Figure 4 quickly reveals that the ingestion routes, because of the first-pass effect, result in the greatest amount of binding and thus the largest relevant internal dose. Also, note that for the two inhalation scenarios the cumulative amounts are nearly equal. The only difference between these two exposure scenarios is the time required to reach the peak, with the peak reached earlier with the higher concentration exposure.

Figure 5 shows the result for the amount bound using model 2. This model is structured to account for clearance of the metabolite from the liver and for the bound fraction to become further incorporated into cellular molecules. Thus, the amounts depicted in Figure 5 are instantaneous amounts rather than cumulative amounts, as were depicted in Figure 4 using Model 1. Again, the ingestion routes show considerably higher levels of bound fractions than the inhalation routes. The repeated ingestion scenario shows significant buildup of the levels with each repeated exposure. Also, the single higher-level oral exposure reaches slightly higher maximum levels than the repeated lower level-oral exposure.

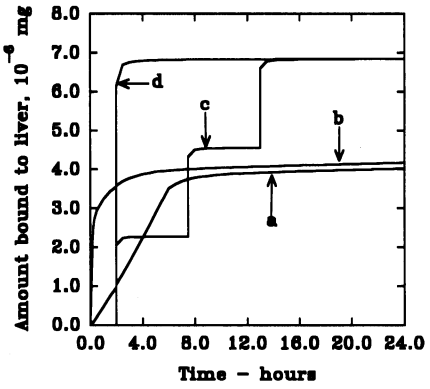

Figure 4. Mass of metabolite bound in liver as estimated by model 1. (*a*) 6-hr inhalation at 0.00035 ppm; (*b*) 10-min inhalation at 0.013 ppm; (*c*) three repeated ingestion doses at 0.0018 mg/l; (*d*) one ingestion dose at 0.0054 mg/l.

Figure 6 shows the results using model 2 for the incorporated concentration. The ingestion routes again result in higher levels. The exact pattern of exposure seems to be less significant than route of exposure. Note that the incorporated concentration shows a continuous rise during the exposure, followed by a decline after the exposure has completely ceased, for the case of repeated ingestion. Although not shown here, the cumulative area under the curve of the incorporated concentration is higher for the ingestion routes. Little difference was observed between each of the two inhalation scenarios, and also between each of the two ingestion routes.

Next, the impact of varying two important hepatic parameters was investigated. In this illustration, both parameters can be affected by concomitant exposure to more than one substance. Metabolic induction is

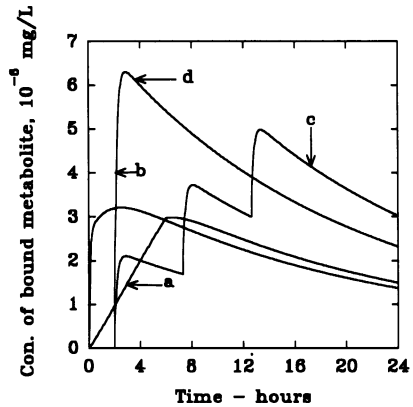

Figure 5. Concentration of bound metabolite in liver as estimated by model 2. (*a*) 6-hr inhalation at 0.00035 ppm; (*b*) 10-min inhalation at 0.013 ppm; (*c*) three repeated ingestion doses at 0.0018 mg/l; (*d*) one ingestion dose at 0.0054 mg/l.

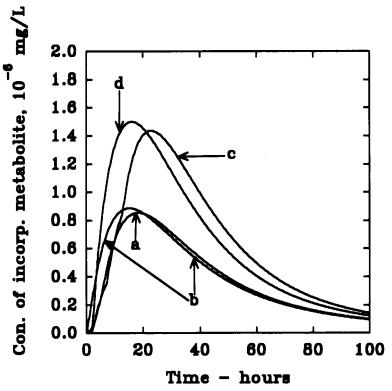

Figure 6. Concentration of incorporated metabolite in liver as estimated by model 2. (*a*) 6-hr inhalation at 0.00035 ppm; (*b*) 10-min inhalation at 0.013 ppm; (*c*) three repeated ingestion doses at 0.0018 mg/l; (*d*) one ingestion dose at 0.0054 mg/l.

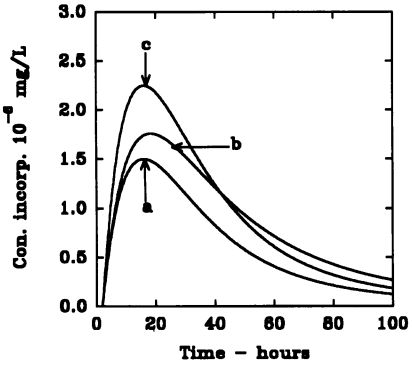

Figure 7. Concentration of incorporated metabolite in liver as estimated by model 2. (*a*) Base line levels for binding capacity and metabolic rate; (*b*) metabolic rate increased by 1.5-fold; (*c*) binding capacity increased by 1.5-fold.

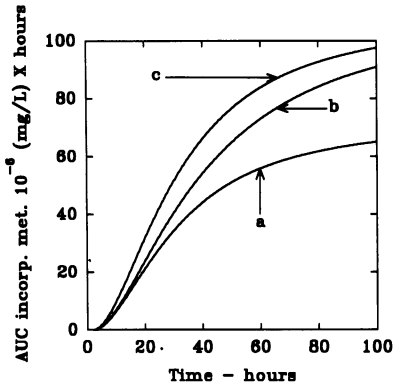

Figure 8. Area under the curve of incorporated metabolite in liver as estimated by model 2. (*a*) Base line levels for binding capacity and metabolic rate; (*b*) metabolic rate increased by 1.5-fold; (*c*) binding capacity increased by 1.5-fold.

known to occur with continued exposure to many chemicals. Often those induced enzymes are then more efficient in metabolizing other xenobiotics. Continued exposure may also induce increased numbers of host sites for binding. This could result from increased protein synthesis or increased cellular proliferation. To make the effects more easily discernible, single exposure through the ingestion route was simulated. Figure 7 shows the results for the incorporated concentration. While both have an effect it can readily be observed that increased binding capacity, for this case, exerts a greater impact on the peak concentration than does increased metabolic rate via induction. However as can be seen by examining Figure 8, the impact on the integrated incorporated concentration is nearly the same for both inducing the enzyme and for increasing the binding capacity of endogenous molecules for the metabolite.

Figures 9 and 10 show the effects of two-step induction of binding capacity when there is repeated daily exposure through ingestion of the compound of interest. By examining the instantaneous concentration (Figure 9) one can readily see at what times the stepwise changes in induction occurred (i.e., at 72 and 168 hr). Note that a new pseudo-steady state is established after each change in the binding capacity. Figure 10 shows the difference between the constant binding capacity case and the induced case. Note that for repeated exposure, increased binding capacity has a greater effect over the long term than it did for the single exposure case (Figure 8). Clearly, the impact of increased binding is far more significant for cases of repeated exposure rather than for single-exposure cases. Also the difference increases with time and repeated exposure. This latter point is very significant if this is the end point to be used in risk assessments where repeated exposure is expected to occur for long periods during the lifetime.

Conclusions

This exercise is illustrative of the power of PBPK models to compare and contrast the relevant toxic internal doses that result from many different exposure scenarios. The contention here is not that a knowledge of pharmacokinetics and pharmacokinetic models is a panacea that will eliminate all uncertainties of the risk assessment process. It is not even a contention that these models will identify all of the uncertainties. Rather, it is contended here that the processes of model formulation,

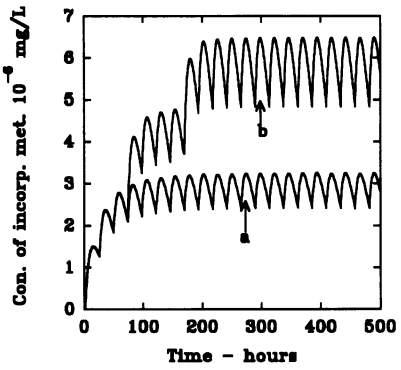

Figure 9. Concentration of incorporated metabolite in liver as estimated by model 2. (*a*) Repeated daily ingestion doses at base line level of binding capacity; (*b*) two-step induction of binding capacity.

validation, and implementation are of great help in making rational risk assessment decisions. This simple illustration concentrated upon pharmacokinetics. Similar approaches need to be taken with pharmacodynamic information. Pharmacokinetic and pharmacodynamic models often require resource-intensive efforts for their proper formulation and validation. However, once formed and validated, they can greatly reduce the costs of risk and exposure assessments. As observed in this illustration, key biologic processes and exposure scenarios can be readily identified. Cost-effective experiments, monitoring, and even amelioration strategies can be designed and implemented. In this example, the exposure route potentially posing the greater risk is identified.

It should be noted that different toxic end points often result in different conclusions regarding concern. As discussed in

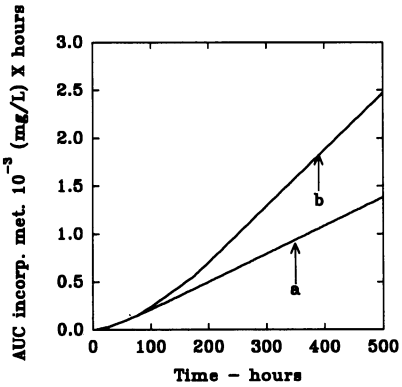

Figure 10. Area under the curve of incorporated metabolite in liver as estimated by model 2. (*a*) Repeated daily ingestion doses at base line level of binding capacity; (*b*) two-step induction of binding capacity.

the previous section, for this illustration, when peak concentration of the parent compound poses the toxic risk, the inhalation route was of greater concern. On the other hand, when some interaction of metabolite with endogenous substances is the toxic mechanism, then the ingestion route poses the greater danger. Similarly, concurrent exposure to other substances may impact at many different points in the physiology and biochemistry of xenobiotic processing. Substances which induce metabolism or those which might increase the number of receptors (e.g., cellular proliferation) clearly impacted the amount of potentially toxic product formed after exposure to xenobiotic, as theorized in this illustration. With adequate knowledge about mechanisms of action, this type of exercise can be and has been performed for a variety of environmental xenobiotics.

Regarding the mechanism, as this illustration has shown, the choice of the end point greatly influences conclusions. The choice of dosimeter is crucial for these more sophisticated risk assessments. Total absorbed dose has frequently been suggested and used by regulatory agencies as a dosimeter of choice for many risk assessments. As has been shown here, identical absorbed masses do not necessarily result in identical levels of internal and more relevant dosimetrics. Erroneous conclusions can easily be reached if the inappropriate dosimetric is chosen. In this case, a number of different endpoints were chosen. Peak concentration of parent, amount of metabolite bound and incorporated were all examined as the potential significant toxic measures. The time integral of incorporated concentration was also examined as one viable risk assessment end point. The exact significance of cumulative measures is not always agreed upon. The history of using cumulative measures in risk assessments is based on the use of some measure of total accumulated dose averaged over some time period, often lifetime. While this may be intuitively attractive for some pathologic processes, care must be exercised so as to appropriately apply such measures. Clearly, the time when dosing or exposure occurs is also crucial. For example, for some chemicals causing fetal toxicity, only doses occurring in specific windows of vulnerability are significant. Cumulative doses over the entire gestation period may be irrelevant. The complex and numerous nonlinearities in pharmacokinetic and pharmacodynamic processes and the time in which they and the exposures occur need to be carefully taken into account.

It is well to remember that modeling and models do not replace well planned and precisely performed laboratory experiments. Rather, they are adjuncts which serve to maximize usefulness of experimental results, assist in the more precise planning of other meaningful experiments, and help design cost-effective and meaningful field monitoring studies. As such, they are tools to help identify and hopefully reduce some of the many uncertainties inherently associated with the risk assessment process. In the end, well-organized assessments based on experimentation, modeling, and realistic monitoring and sound scientific judgment will result in rational and effective risk management decisions.

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